Reliable Identification of Living Dopaminergic Neurons in Midbrain Cultures Using RNA Sequencing and TH-promoter-driven eGFP Expression.

Journal: J Vis Exp

Publication Year: 2017

Authors: Beverley M Henley, Bruce N Cohen, Charlene H Kim, Heather D Gold, Rahul Srinivasan, Sheri McKinney, Purnima Deshpande, Henry A Lester

PubMed link: 28287593

Funding Grants: Bridges to Stem Cell Research at Pasadena City College

Public Summary:
In Parkinson's Disease (PD) there is widespread neuronal loss throughout the brain with pronounced degeneration of dopaminergic neurons in the SNc, leading to bradykinesia, rigidity, and tremor. The identification of living dopaminergic neurons in primary Ventral Mesencephalic (VM) cultures using a fluorescent marker provides an alternative way to study the selective vulnerability of these neurons without relying on the immunostaining of fixed cells. Here, we isolate, dissociate, and culture mouse VM neurons for 3 weeks. We then identify dopaminergic neurons in the cultures using eGFP fluorescence (driven by a Tyrosine Hydroxylase (TH) promoter). Individual neurons are harvested into microcentrifuge tubes using glass micropipettes. Next, we lyse the harvested cells, and conduct cDNA synthesis and transposon-mediated "tagmentation" to produce single cell RNA-Seq libraries(1)(,)(2)(,)(3)(,)(4)(,)(5). After passing a quality-control check, single-cell libraries are sequenced and subsequent analysis is carried out to measure gene expression. We report transcriptome results for individual dopaminergic and GABAergic neurons isolated from midbrain cultures. We report that 100% of the live TH-eGFP cells that were harvested and sequenced were dopaminergic neurons. These techniques will have widespread applications in neuroscience and molecular biology.

Scientific Abstract:
In Parkinson's Disease (PD) there is widespread neuronal loss throughout the brain with pronounced degeneration of dopaminergic neurons in the SNc, leading to bradykinesia, rigidity, and tremor. The identification of living dopaminergic neurons in primary Ventral Mesencephalic (VM) cultures using a fluorescent marker provides an alternative way to study the selective vulnerability of these neurons without relying on the immunostaining of fixed cells. Here, we isolate, dissociate, and culture mouse VM neurons for 3 weeks. We then identify dopaminergic neurons in the cultures using eGFP fluorescence (driven by a Tyrosine Hydroxylase (TH) promoter). Individual neurons are harvested into microcentrifuge tubes using glass micropipettes. Next, we lyse the harvested cells, and conduct cDNA synthesis and transposon-mediated "tagmentation" to produce single cell RNA-Seq libraries(1)(,)(2)(,)(3)(,)(4)(,)(5). After passing a quality-control check, single-cell libraries are sequenced and subsequent analysis is carried out to measure gene expression. We report transcriptome results for individual dopaminergic and GABAergic neurons isolated from midbrain cultures. We report that 100% of the live TH-eGFP cells that were harvested and sequenced were dopaminergic neurons. These techniques will have widespread applications in neuroscience and molecular biology.